

**CLAIMS:**

1. An enriched preparation of human undifferentiated embryonic stem  
5 cells wherein said cells are capable of proliferation *in vitro* and  
differentiation to neural progenitor cells, neuron cells and/or glial cells.
2. The enriched preparation of human undifferentiated embryonic stem  
cells according to claim 1 wherein said cells maintain an undifferentiated  
10 state when cultured on a fibroblast feeder layer in the absence of a  
differentiating signal.
3. The enriched preparation of human undifferentiated embryonic stem  
cells according to claim 1 or 2 wherein said cells are capable of  
15 differentiation into neural progenitor cells.
4. An undifferentiated human embryonic stem cell wherein the cell is  
capable of proliferation *in vitro* and differentiation to neural progenitor cells,  
neuron cells and/or glial cells and is immunoreactive with markers for human  
20 pluripotent stem cells including SSEA-4, GCTM-2 antigen, and TRA 1-60.
5. The undifferentiated human embryonic stem cell according to claim 4  
wherein the cell expresses Oct-4.
- 25 6. The undifferentiated human embryonic stem cell according to claim 5  
wherein said cell maintains a diploid karyotype during prolonged cultivation  
*in vivo*.
7. The undifferentiated human embryonic stem cell according to any one  
30 of claims 4 to 6 which forms tumors when injected in the testis of  
immunodeprived SCID mice.

8. A differentiated committed human progenitor cell line capable of differentiation and propagation into mature neurons and/or glial cells said cell line derived from undifferentiated human embryonic stem cells.

5 9. The differentiated committed human progenitor cell line according to claim 8 capable of establishing a graft in a recipient brain.

10 10. The differentiated committed human progenitor cell line according to claim 9 capable of differentiating *in vivo* into other cell lineages including neurons and glial cells wherein the glial cells are selected from the group including astrocytes and oligodendrocytes.

11. A neural progenitor cell differentiated *in vitro* from an undifferentiated human embryonic stem cell.

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12. The neural progenitor cell according to claim 11 wherein said cell is capable of proliferation.

13. The neural progenitor cell according to claim 11 wherein said cell is capable of differentiating to a mature neuron cell or glial cell.

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14. The neural progenitor cell according to claim 11 wherein said cell is capable of transdifferentiation into other cell lineages to generate stem cells and differentiated cells of non-neural phenotype including hemangioblast, haematopoietic stem cells, endothelial stem cells, embryonic endoderm and ectodermal cells.

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15. The differentiated neural progenitor cell according claims 8 or 11 characterised by expressed markers including markers of the neuroectodermal lineage; markers of neural progenitor cells; neuro-filament proteins; monoclonal antibodies including MAP2ab; glutamate; synaptophysin; glutamic acid decarboxylase; GABA, serotonin, tyrosine

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hydroxylase;  $\beta$ -tubulin;  $\beta$ -tubulin III; GABA A $\alpha$ 2 receptor, glial fibrillary acidic protein (GFAP), , 2', 3'- cyclic nucleotide 3'- phosphodiesterase (CNPase), *p/p*, DM-20 ,O4 and NG-2 immunostaining.

5     16.     The neural progenitor cell according to claim 15 which expresses markers of neuroectoderm and neural progenitor cells selected from the group including polysialylated N-CAM, N-CAM, A2B5, nestin, vimentin and the transcriptional factor Pax-6, and do not express Oct-4.

10     17.     The neural progenitor cell according to claim 16 wherein said cell is capable of establishing a graft in a recipient brain.

18.     The neural progenitor cell according to claim 17 wherein said cell can incorporate extensively into a recipient brain.

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19.     The neural progenitor cell according to claim 18 wherein said cell is capable of migrating along host brain pathways.

20     20.     The neural progenitor cell according to claim 19 wherein said cell is capable of wide spread distribution in host brain.

21.     The neural progenitor cell according to claim 20 wherein said cell is responsive to host environmental signals.

25     22.     The neural progenitor cell according to claim 21 wherein said cell differentiates in response to local host environmental signals.

23.     The neural progenitor cell according to claim 22 wherein said cell is capable of differentiation to progeny of neural lineages selected from the  
30     group including neurons, oligodendrocyte and astrocyte in a recipient brain.

24. The enriched preparation of neural progenitor cells including an enrichment of cells according to claim 23.

25. The enriched preparation of neural progenitor cells according to claim  
5 24 wherein said cells are capable of prolonged undifferentiated proliferation and expansion in *in vitro* culture.

26. The enriched preparation of neural progenitor cells according to claim  
10 24 wherein said cells are capable of differentiation into neurons, mature neurons and glial cells.

27. The enriched preparation of neural progenitor cells according to claim  
15 24 wherein said cells are capable of establishing a graft in a recipient brain in the absence of tumors.

28. The enriched preparation of neural progenitor cells according to claim  
25 wherein said cells may be recovered from cryopreservation.

29. A method of preparing undifferentiated human embryonic stem cells  
20 for differentiation into neural progenitor cells, said method including:  
obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;  
removing inner cells mass (ICM) cells from the embryo;  
culturing ICM cells under conditions which do not induce  
25 extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; and  
recovering the stem cells.

30. The method according to claim 29 including culturing the ICM cells on  
30 a fibroblast feeder layer to promote proliferation of embryonic stem cells prior to recovering the stem cells from the feeder layer.

31. The method according to claim 30 wherein said fibroblasts are selected from human or mouse fibroblasts or a combination of human and mouse fibroblasts.

5 32. The method according to claim 31 wherein the fibroblast feeder layer comprises embryonic fibroblasts.

33. The method according to claim 32 wherein said fibroblasts are derived from inbred 129/Sv or CBA mice or mice from a cross of 129/Sv with  
10 C57/B16 strains.

34. The method according to claim 30 wherein said fibroblast feeder layer has a density of approximately 25,000 human and 70,000 mouse cells per  $\text{cm}^2$  or 75,000 to 100,000 mouse cells per  $\text{cm}^2$ .

15 35. The method according to claim 34 wherein the fibroblast feeder layer is established 6 to 48 hours prior to addition of ES or ICM cells.

36. The method according to claim 35 wherein the fibroblast feeder cells  
20 are arrested in their growth.

37. The method according to claim 36 wherein the fibroblast feeder cells are arrested by irradiation or treated with mitomycin C.

25 38. The method according to claims 29 further including: replating the stem cells from the fibroblast feeder layer onto another fibroblast feeder layer; and  
culturing the stem cells for a period sufficient to promote proliferation of morphologically undifferentiated stem cells.

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39. The undifferentiated human embryonic stem cell prepared by a method according to claim 38.

40. A method of inducing somatic differentiation of stem cells *in vitro* into progenitor cells said method comprising:

- obtaining undifferentiated embryonic stem cells; and
- 5 providing a differentiating signal under conditions which are non-permissive for stem cell renewal, do not kill cells and/or induces unidirectional differentiation toward extraembryonic lineages.

41. The method according to claim 40 wherein said undifferentiated  
10 embryonic stem cell is an undifferentiated human embryonic stem cell wherein the cell is capable of proliferation *in vitro* and differentiation to neural progenitor cells, neuron cells and/or glial cells and is immunoreactive with markers for human pluripotent stem cells including SSEA-4, GCTM-2 antigen, and TRA 1-60.

15 42. The method according to claim 40 wherein the conditions for inducing somatic differentiation of stem cells are selected from any one of the following including:

- culturing the undifferentiated stem cells for prolonged periods and at  
20 high density on a fibroblast feeder cell layer to induce differentiation;
- culturing the undifferentiated stem cells in serum free media;
- culturing the undifferentiated stem cells on a differentiation inducing fibroblast feeder layer and wherein said fibroblast feeder layer does not induce extra embryonic differentiation and cell death;
- 25 culturing to a high density in monolayer or on semi-permeable membranes so as to create structures mimicing the postimplantation phase of human development; or
- culturing in the presence of a chemical differentiation factor selected from the group including bone morphogenic protein-2 or antagonists thereof.

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43. A differentiated progenitor cell prepared by the method according to any one of claims 40 to 42.

44. The differentiated progenitor cell according to claim 43 selected from the group consisting of a neural progenitor cell, mesodermal progenitor cell including hemangioblast, hematopoietic or endothelial stem cells,  
5 endodermal and ectodermal progenitors.

45. The differentiated progenitor cell according to claim 44 which is a neural progenitor cell capable of differentiating into a neuron cell and/or a glial cell.  
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46. A method of inducing somatic cells from embryonic stem cell derived somatic progenitors, said method comprising:  
obtaining a source of embryonic stem cell derived somatic  
progenitors; culturing the progenitor cells on an adhesive substrate; and  
15 inducing the cells to differentiate to somatic cells under conditions which favour somatic differentiation.

47. The method according to claim 46 wherein said embryonic stem cell derived somatic progenitor cells are grown in the presence of a serum free  
20 media and growth factors and are induced to differentiate by withdrawal of the growth factors.

48. The method according to claim 46 wherein the embryonic stem cell-derived progenitor cell is a cell selected from the group consisting of a neural  
25 progenitor cell, mesodermal progenitor cell including hemangioblast, hematopoietic or endothelial stem cells, endodermal and ectodermal progenitors.

49. The method according to claim 46 wherein the progenitor cells are  
30 cultured on an adhesive substrate selected from poly-D-lysine and laminin or poly-D-lysine and fibronectin.

50. The method according to claim 49 wherein the progenitor cells are cultured on poly-D-lysine and laminin.

51. The method according to claim 50 wherein the cells are further  
5 cultured in the presence of retinoic acid.

52. The method according to claim 47 wherein said somatic cells induced are neurons including mature neurons.

10 53. A mature neuron cell prepared by the method according to claim 52 and characterised by expression of the 160kDa neurofilament protein, MAP2ab, glutamate, synaptophysin, glutamic acid decarboxylase (GAD), GABA, tyrosine hydroxylase and serotonin.

15 54. The method according to claim 49 wherein the progenitor cells are cultured on poly-D-lysine and fibronectin.

55. The method according to claim 54 wherein the progenitor cells are cultured before and after plating on poly-D-lysine and fibronectin in serum  
20 free medium in the presence of PDGF-AA and bFGF.

56. The method according to claim 55 wherein the progenitor cells are cultured after plating in the presence of PDGF-AA, basic FGF and EGF .

25 57. The method according to claim 56 further including culturing the somatic progenitor cells after plating in the presence of T3.

58. The method according to claim 57 wherein said somatic cells induced are glial cells including astrocyte and oligodendrocyte cells.

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59. An oligodendrocyte cell prepared by the method according to claim 58 and characterized by RNA transcription of MBP, *plp*, *dm-20* and immunostaining for O4 and NG-2.

5 60. A method of producing an enriched preparation of human ES derived neural progenitor cells, said method comprising:

obtaining an undifferentiated human embryonic stem cell according to claim 39;

10 inducing somatic differentiation of the embryonic stem cell to a neural progenitor cell by a method according to claim 40;

identifying a neural progenitor cell by expressed markers of primitive neuroectoderm and neural stem cells and wherein said markers are selected from the group including polysialylated N-CAM, N-CAM, A2B5, intermediate filament proteins including nestin and vimentin and the transcription factor

15 Pax-6; and

culturing the neural progenitor cells to promote proliferation and propagation.

20 61. The method according to claim 60 wherein the neural progenitor cells are cultured as spheres or monolayers in serum free medium comprising DMEM/F12 supplemented with B27 and growth factors.

25 62. The method according to claim 61 wherein the growth factors include EGF and bFGF.

63. The method according to claim 62 including further culturing to eliminate non-neural cells, in particular extrabryonic endodermal cells, said culturing comprising further selective culturing in serum free media including DMEM/F12 supplemented with B27 and growth factors.

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64. The method according to claim 63 wherein the further culturing includes the transfer of undifferentiated ES cell clumps into serum free

medium comprised of DMEM/F12 supplemented with B27, bFGF and EGF and cultivation of the resulting neural progenitors as spheres or monolayers.

65. A method of transplanting ES derived neural progenitor cells in a  
5 host, said method comprising:

obtaining a source of neural progenitor cells prepared by a method according to claims 40;

culturing the neural progenitor cells in the presence of serum free medium supplemented with B27 and growth factors including, EGF and  
10 bFGF; and

injecting the neural progenitor cells into the nervous system of the host.

66. The method according to claim 65 wherein the neural progenitor cells  
15 are injected into the lateral cerebral ventricle of the nervous system.

67. A method of producing a stable graft of neural cells and contributing in the histogenesis of a living host said method comprising:

transplanting ES derived neural progenitor cells into a living host by a  
20 method according to claim 66.

68. A method of modifying a nervous system of a host, said method comprising transplanting ES derived neural progenitor cells by a method according to claims 67.  
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69. The method according to claim 68 wherein said modifying of the nervous system includes any one of replacing deficient neuronal or glial cell populations, restoring deficient functions or activating regenerative and healing processes in the nervous system to regenerate cell populations.  
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70. The method according to claim 68 wherein the neural progenitor cells comprise genetically modified neural progenitor cells.

71. The method according to claim 70 wherein the genetically modified neural progenitor cells express specific desired genes at the target organ.

5 72. A method for treating a pathological condition of the nervous system comprising modifying a nervous system of a patient according to claims 68.

73. The method according to claim 72 wherein the pathological condition  
10 is selected from the group including neurodegenerative disorders, mental disorders, vascular conditions, autoimmune disorders, congenital disorders, and trauma.